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Research Article

A Comparative Study on the Effectiveness of *Azotobacter chroococcum* and *Beijerinckia indica* as Biofertilizers for Enhancing the Growth of *Alternanthera ficoidea*

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Abstract

The increasing global demand for food has driven the development of environmentally friendly technologies in agriculture, one of which involves the use of nitrogen-fixing bacteria-based biofertilizers. This study aims to evaluate the effectiveness of two nitrogen-fixing bacterial species, Azotobacter chroococcum and Beijerinckia indica, in enhancing the growth of red spinach (Alternanthera ficoidea) in acidic soil conditions. The results revealed that treatments with various concentrations of bacterial inoculants did not have a statistically significant effect on plant height, based on a two-way ANOVA test (α > 0.05). However, descriptively, B. indica demonstrated more optimal performance compared to A. chroococcum and their combination, particularly at a concentration of 15 mL, which produced the highest plant height (31.00 cm). B. indica exhibited better survival and functionality in acidic soil (pH 4–4.5), whereas A. chroococcum was less efficient under such conditions. These findings underscore the importance of selecting bacterial strains that are compatible with specific soil characteristics to enhance the efficacy of biofertilizers and support the growth of ornamental plants such as A. ficoidea.

Keywords: *Azotobacter chroococcum; Beijerinckia indica; Biofertilizers; Effectiveness.*

Introduction

The rapid increase in global food demand due to population growth necessitates serious attention in the agricultural sector [1], [2]. One widely adopted solution to improve agricultural productivity is the use of environmentally friendly fertilizer technologies, particularly biofertilizers. Biofertilizers are fertilizers containing living microorganisms that can directly or indirectly promote plant growth [3]. The use of biofertilizers not only reduces dependence on chemical fertilizers—which can cause environmental damage—but also enhances soil quality and plant resistance to various diseases [4], [5].

A widely developed type of biofertilizer involves nitrogen-fixing bacteria, as nitrogen is an essential nutrient for protein synthesis and vegetative growth in plants [6]. One of the most commonly used bacteria in biofertilizers is *Azotobacter chroococcum*, a free-living nitrogen-fixing bacterium known for increasing nitrogen availability in soil, particularly in nitrogen-deficient conditions [7], [8]. Similarly, *Beijerinckia indica* also possesses nitrogen-fixing capabilities and contributes to soil quality improvement through biological activity that enhances soil structure and increases the availability of other essential nutrients [7], [9]. The use of nitrogen-fixing bacterial biofertilizers has been shown to significantly improve agricultural yields [10]. These bacteria help convert atmospheric nitrogen into a form that can be absorbed by plants, thereby promoting better growth [11]. One plant species that could benefit from the application of biofertilizers is *Alternanthera ficoidea*, a species with various beneficial properties.

Alternanthera ficoidea is a plant species with significant potential in the field of pharmacology. Research has shown that species within the Alternanthera genus contain approximately 129 identified chemical compounds, including flavonoids, steroids, saponins, alkaloids, triterpenoids, glycosides, and phenolic compounds [12]. Despite its considerable potential as both an ornamental plant and a source of various bioactive compounds, the utilization of *A. ficoidea* among the general public remains relatively limited. It is primarily recognized as an ornamental plant used to beautify gardens or open spaces, with little awareness of its broader benefits. To achieve optimal growth and high plant quality, proper cultivation practices—including effective fertilization—are essential. Therefore, investigating the application of *Azotobacter chroococcum* and *Beijerinckia indica* as biofertilizers to support the growth of *Alternanthera ficoidea* is of great importance, particularly in determining which bacterial species is most effective in enhancing the productivity of this ornamental plant.

Materials and Methods

The materials used in this study included *Azotobacter chroococcum* and *Beijerinckia indica* bacterial strains, red spinach (*Alternanthera ficoidea*) seeds, distilled water (aquadest), aluminum foil, cotton, *Nutrient Agar* (NA), and *Nutrient Broth* (NB) media.

1. *Planting Media Preparation*

Soil was collected from a plantation area, then processed by crushing and sieving it using a 5 mm mesh sieve. A 25-gram soil sample was taken for analysis of its properties, which included measuring soil pH using a pH meter and identifying the soil type based on its color and physical characteristics.

2. *Preparation of Nutrient Broth and Nutrient Agar Media*

Nutrient Broth (NB) medium was prepared by dissolving 1.2 g of NB powder into 150 mL of distilled water in a beaker, followed by heating on an electric stove until completely dissolved. The medium was then distributed into test tubes (5 mL per tube), sealed with cotton plugs, and covered with aluminum foil. The tubes were sterilized using an autoclave at 1 atm pressure and 121 °C for 15–20 minutes. For the preparation of *Nutrient Agar* (NA), 2 g of NA powder was mixed with 100 mL of distilled water and heated until fully dissolved. The solution was then transferred into test tubes and sterilized using the same autoclaving procedure as for the *Nutirent Broth* (NB) medium. After sterilization, the test tubes were stored in a slanted position to allow the agar to solidify properly [13].

3. *Bacterial Isolate Propagation of A. chroococcum and B. indica*

Following isolate rejuvenation, bacterial propagation was conducted as follows: two loopfuls of *Azotobacter chroococcum* isolate were taken from slanted NA medium and cultured in 150 mL of Nutrient Broth. Similarly, two loopfuls of *Beijerinckia indica* isolate were inoculated into 150 mL of Nutrient Broth. Additionally, a mixed culture was prepared by inoculating two loopfuls of each isolate (*A. chroococcum* and *B. indica*) into 150 mL of Nutrient Broth. The bacterial cultures were incubated for 24 hours before being inoculated into the soil [14].

4. Bacterial Inoculation into Soil

After rejuvenation and propagation, the bacterial inoculants were introduced into approximately 1 kg of soil contained in polybags, according to the specified treatments. Inoculation was performed following the sowing of red spinach (*Alternanthera ficoidea*) seeds [15].

5. Sowing of Red Spinach (Alternanthera ficoidea) Seeds

Five seeds of *Alternanthera ficoidea* were sown in each polybag containing approximately 1 kg of soil. The plants were watered using a sprayer and allowed to grow for 2–3 days. Growth observations began 2–3 days after planting and included weekly measurements of plant height (in cm) and leaf number. Watering was adjusted according to seasonal conditions—once daily during the dry season and reduced during the rainy season [16]. Harvesting was carried out after 49 days (7 weeks).

6. Data Analysis

The data were analyzed using two-way ANOVA to determine the statistical significance of treatment effects on plant height and leaf number. A significance level of $\alpha = 0.05$ was used. The data obtained from plant growth observations, including leaf count and plant height, were recorded weekly from Week 1 to Week 7.

Results and Discussion

The application of different bacterial treatments—*A. chroococcum*, *B. indica*, and a combination of both—did not show a statistically significant effect on the growth of red spinach plants. Nevertheless, the findings provide insight into which bacterial strain may be more effective in enhancing plant growth. Statistical analysis of the effect of bacterial type on plant height yielded a significance value of $\alpha = 0.554$ ($\alpha > 0.05$), indicating that the plant height data were normally distributed. Furthermore, the results of the two-way ANOVA test showed no significant differences in plant height among the bacterial treatments, with a significance value of $\alpha = 0.528$ ($\alpha > 0.05$). However, as shown in Table 1, *B. indica* appeared to be more effective in promoting plant height, with an average height of 26.75 cm.

Tabel 1. Average Plant Height of *Alternanthera ficoidea* Under Different Bacterial Treatments

Bacteria	Concentration (mL)	Height (cm)
A. chroococcum	0	23.75 ± 0.5
	10	23.33 ± 1.5
	15	27.33 ± 6.6
	20	25.00 ± 2.6
	Average	**24.85 ± 3.70**
B. indica	0	22.33 ± 7.5
	10	26.33 ± 5.5
	15	31.00 ± 2.6
	20	27.33 ± 3.2
	Average	**26.75 ± 5.41**
Combination isolate Bacteria *A. chroococcum+B. indica*	0	24.00 ± 4.3
	10	24.33 ± 7.5
	15	30.00 ± 1.0
	20	22.67 ± 3.7
	Average	**25.25 ± 5.01**

The addition of nitrogen to soil plays a critical role in accelerating plant height growth [17]. In this context, *Beijerinckia indica* was found to be more effective in enhancing plant height compared to *Azotobacter chroococcum*. Although *A. chroococcum* is a well-known free-living nitrogen-fixing bacterium with a strong capacity for nitrogen fixation [18], the results of this study indicated that *B. indica* was superior in promoting plant growth. The key factor influencing this outcome is the acidic nature of the soil (pH 4–4.5) in which the plants were grown. *A. chroococcum* exhibits optimal growth in neutral soil conditions, with a preferred pH range of 7.0–7.5 [19], [20].

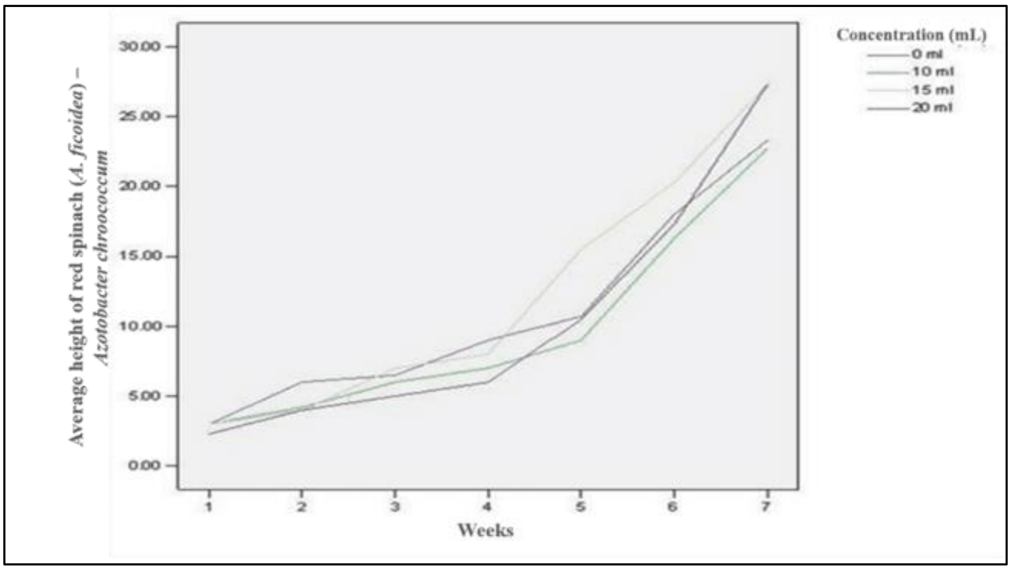

Figure 1. Growth Chart of Red Spinach (*A. ficoidea*) Leaf Height with the Application of *Azotobacter chroococcum.*

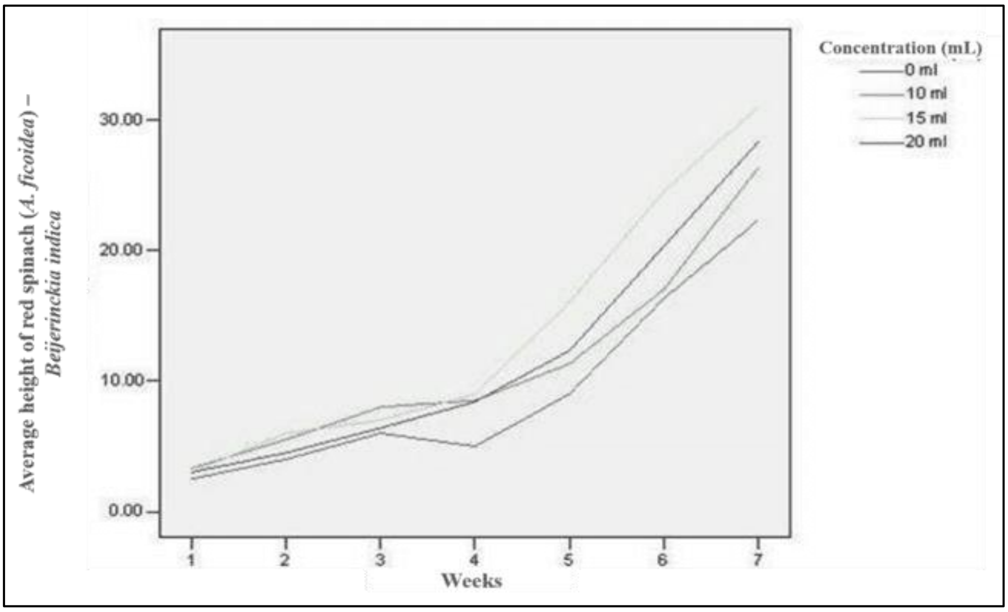

Figure 2. Growth Chart of Red Spinach (*A. ficoidea*) Leaf Height with the Application of *Beijerinckia indica*.

In contrast, *B. indica* is an aerobic bacterium capable of surviving and thriving in acidic soils [9], [21], allowing it to fix nitrogen effectively under such conditions, thereby supporting the growth of red spinach (*Alternanthera ficoidea*). The enhanced performance of *B. indica* in acidic soil underscores the importance of understanding microbial-soil interactions and their implications for plant development. Further research on the performance of *B. indica* under various acidic soil conditions could provide valuable insights for the application of biofertilizers in improving crop yields. This also highlights the potential of *B. indica* as a more pH-resilient biofertilizer solution compared to *A. chroococcum*, which is limited to neutral pH environments.

The effect of bacterial type combinations at various concentrations on plant height yielded a significance value of 0.590 ($p>0.05$), indicating that the plant height data were normally distributed. Based on the results of the two-way ANOVA test, bacterial type did not have a statistically significant effect on plant height, with a significance value of $\alpha = 0.023$ ($\alpha>0.05$). The two-way ANOVA analysis for the interaction between bacterial combinations and concentration levels also showed no significant differences. However, treatment with *B. indica* at a concentration of 15 mL resulted in the highest average plant height (31.00 cm) (Table 1). In the treatment with a combination of A. chroococcum and B. indica at various concentrations, no significant differences were observed in enhancing the growth of red spinach plants. Nevertheless, the results indicate an improvement in both plant height and leaf number parameters of Alternanthera ficoidea with the application of the combination of both bacterial strains at different concentrations. The application of A. chroococcum at various concentrations showed differences in the rate of growth observed weekly (Figures 1 and figure 2).

In the first week, plant height growth appeared almost uniform, but differences in growth rates began to emerge in the second week. By the seventh week, the control group showed more distinct differences in growth compared to the bacterial treatments. At the seventh week, the application of *A. chroococcum* at concentrations of 15 mL and 20 mL resulted in similar growth rates, with maximum growth ranging from 27 to 30 cm.

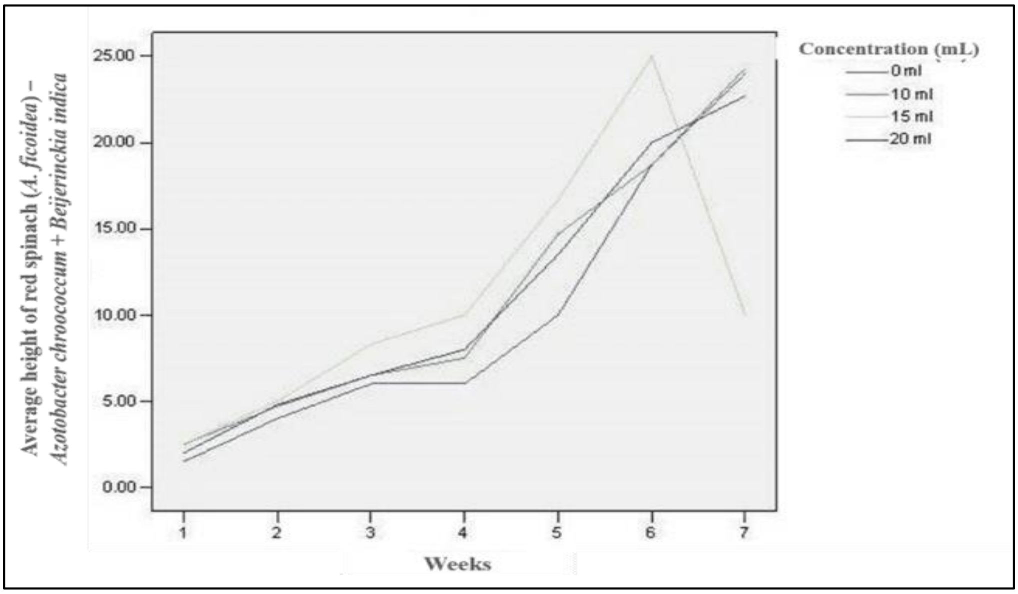

Figure 3. Growth Chart of Red Spinach (*A. ficoidea*) Leaf Height with the Application Combination of *Azotobacter chroococcum* and *Beijerinckia indica*.

The application of *B. indica* at various concentrations showed relatively uniform plant growth in the first week. By the second week, the 15 mL concentration of *B. indica* exhibited more optimal results in enhancing plant height compared to the other treatments, and this effect persisted through the seventh week. At the seventh week (harvest), the optimal growth for plant height was achieved with the 15 mL concentration, followed by 20 mL, 10 mL, and the control group. The 15 mL concentration at week seven resulted in plant heights exceeding 30 cm, while the other concentrations ranged between 20 to 30 cm (Figures 3 and figure 4).

Figure 4. Comparison of Red Spinach (*Alternanthera ficoidea*) Plant Height at Harvest with the Application of *Azotobacter chroococcum* Bacteria at Various Concentrations: a) 0 mL, control; b) 10 mL; c) 15 mL; d) 20 mL.

The application of a combination of *A. chroococcum* and *B. indica* at various concentrations also showed significant differences between treatments. In the sixth week, the 15 mL concentration demonstrated highly optimal plant height growth compared to the other treatments. However, by the seventh week, plant growth at the 15 mL concentration sharply declined. Meanwhile, at the 10 mL and 20 mL concentrations, plant growth remained relatively stable and gradually increased each week. Nevertheless, by the seventh week, growth at the 20 mL concentration began to slow down compared to the 0 mL and 10 mL concentrations (Figures 5 and figure 6).

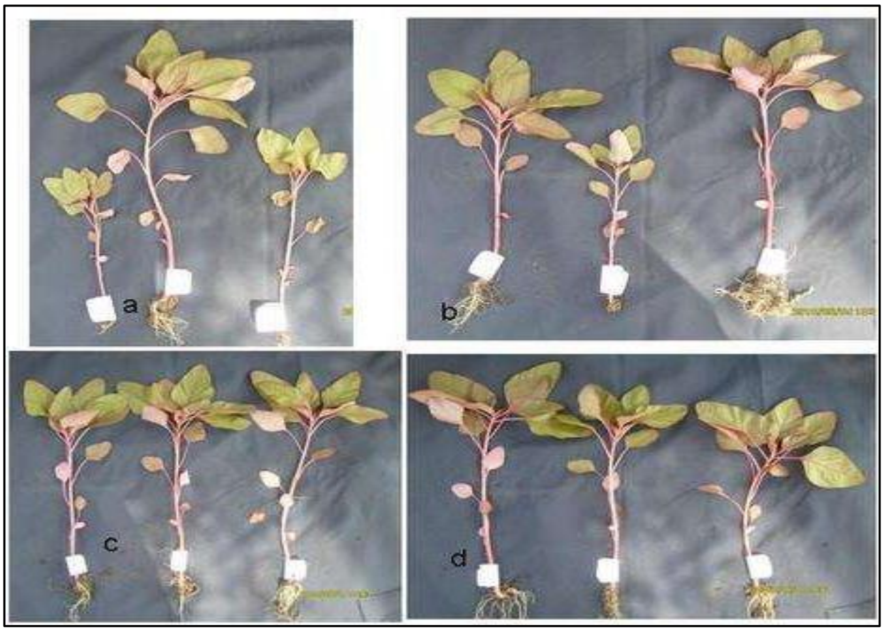

Figure 5. Comparison of Red Spinach (*Alternanthera ficoidea*) Plant Height at Harvest with the Application of *Beijerinckia indica* Bacteria at Various Concentrations: a) 0 mL, control; b) 10 mL; c) 15 mL; d) 20 mL.

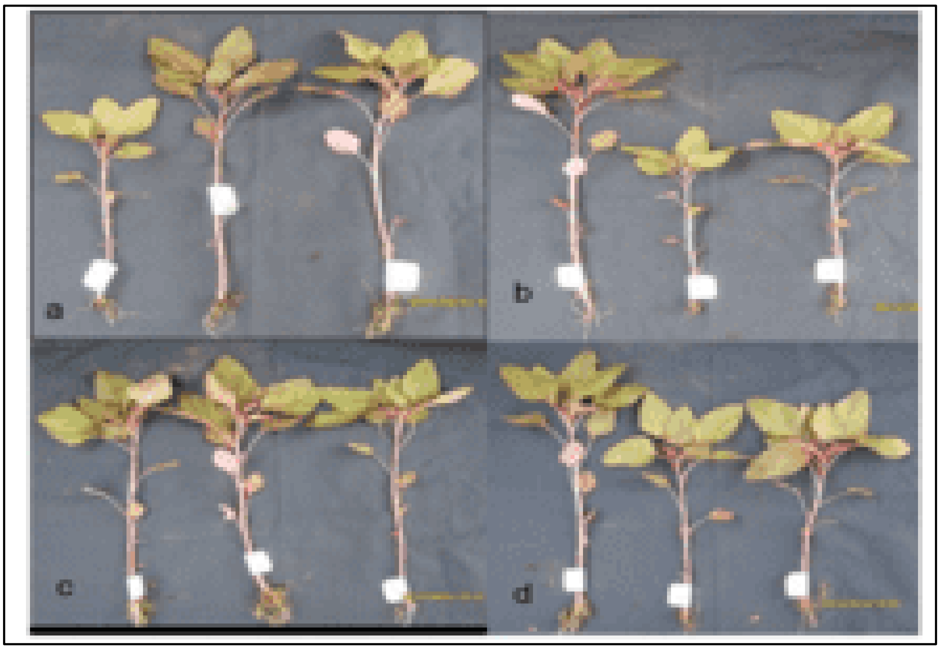

Figure 6. Comparison of Red Spinach (*Alternanthera ficoidea*) Plant Height at Harvest with the Application of a Mixture of *Azotobacter sp.* and *Beijerinckia sp.* Bacteria at Various Concentrations: a) 0 mL; b) 10 mL; c) 15 mL; d) 20 mL.

The addition of nitrogen-fixing bacterial inoculants has been shown to significantly enhance nitrogen availability in the soil, which is one of the essential nutrients for plants, particularly in supporting vegetative growth such as increased plant height [22]. Nitrogen plays a crucial role in protein and chlorophyll synthesis, making its availability highly influential in the rate of photosynthesis and overall plant growth [23], [24]. In the context of acidic soils, selecting the right nitrogen-fixing bacteria is critical because not all bacteria can survive and function optimally in low pH conditions. *Beijerinckia indica* has been shown to have a high tolerance for acidic soils and can remain active in nitrogen fixation under such conditions [25].

Azotobacter chroococcum, although also an effective nitrogen-fixing bacterium in neutral to slightly alkaline soils, exhibits lower efficiency in soils with very low pH, such as the growing medium used in this study (pH 4–4.5). This is consistent with other studies which indicate that the effectiveness of *A. chroococcum* in enhancing plant growth tends to decrease in soils with a pH below 5 [26]. The combination of both bacteria did not yield better results than the sole use of *B. indica*, which is likely due to microorganism competition in the rhizosphere, as well as possible antagonistic interactions or ecological incompatibility between the bacterial species [26]. Therefore, selecting the appropriate bacterial species that matches the soil characteristics is crucial for optimal nitrogen fixation efficiency, ultimately leading to positive effects on plant growth, such as in red spinach (*Alternanthera ficoidea*).

Conclusion

The study showed that the application of *Azotobacter chroococcum*, *Beijerinckia indica*, or their combination did not have a statistically significant effect on the growth height of *Alternanthera ficoidea*. However, *B. indica* was more effective descriptively, particularly at a concentration of 15 mL, which resulted in the highest growth.

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